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The category of products known as dietary supplements covers a wide range of products including multivitamins, herbal supplements and energy drinks. High performance liquid chromatography (HPLC) is a common analytical technique for pharmaceuticals. This study presents an HPLC method for the quantitation of several ingredients in dietary supplements Lentra, Prolent, and Somni-TR.

Photodetection by polydiacetylene is another quantitative method. Polydiacetylenes have two optical states, a “red” and a “blue” state, and an irreversible transition can be triggered from the blue to the red state by the binding of an analyte to the polymer. The potential usefulness of PDA lies in its ability to act as both receptor and transducer. In this study, attempts were made to synthesize several diacetylene monomers which could be used in PDA thin films or vesicles. These attempts included several different chain lengths and functional groups.

A METHOD FOR THE DETERMINATION OF THE INGREDIENTS OF SEVERAL DIETARY SUPPLEMENTS BY LIQUID CHROMATOGRAPHY

by

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Approved by

_____
Committee Chair

To my assorted family

and especially

to Nathan and Penny

APPROVAL PAGE

This thesis has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

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CHAPTER I

INTRODUCTION

Statement of Problem

Dietary or nutritional supplements are defined as a product taken orally and containing ingredients supposed to supplement the diet. The business of selling such supplements is large and growing, with sale of herbal supplements alone growing from $4.8 billion to $5.0 billion between 2008 and 2009.[1] The FDA has placed these products in a gray area as a subcategory of food although many substances categorized as dietary supplements come in tablet or capsule form. The legal definition of a dietary supplement is:

> A product (other than tobacco) intended to supplement the diet that bears or contains one or more of the following dietary ingredients: (A) a vitamin; (B) a mineral; (C) an herb or other botanical; (D) an amino acid; (E) a dietary substance for use by man to supplement the diet by increasing the total dietary intake; or (F) a concentrate, metabolite, constituent, extract, or combination of any ingredient described in clause (A), (B), (C), (D), or (E)[2]

According to the 1994 Dietary Supplement Health and Education Act, the safety of dietary supplements and the accuracy of advertising claims has been the responsibility of the manufacturer and distributor and requires no FDA approval, unlike other food products.[3] Since the manufacturer alone is responsible for the accuracy of the product

label information, it is important for distributors to develop their own methods of quality control.

Lentra, Prolent, and Somni-TR are supplements intended to correct neurotransmitter imbalance by inhibitory support. Each is a formula of ingredients chosen for their complementary roles in affecting neurological systems. The following are the manufacturer's claims regarding their product[1]. Prolent and Lentra are inhibitory formulas for the hypothalamic-pituitary-adrenal (HPA) axis, which is the part of the neuroendocrine system which regulates the body's chemical reactions to stress. Over-excitation of the HPA axis can cause anxiety and sleeplessness, and by boosting an inadequate inhibitory system these symptoms can be treated. Prolent supports the inhibitory system in a variety of ways, including 5-hydroxytryptophan (5-HTP), a serotonin and melatonin precursor, and Suntheanine (a commercial preparation of L-theanine), an agonist for *gamma*-aminobutyric acid (GABA), the major inhibitory neurotransmitter[4]. Lentra acts as direct support for the GABA system with taurine (a GABA receptor agonist), and L-theanine. Somni-TR is a sleep support formula. It utilizes melatonin, a hormone which is normally produced in the pineal gland and acts as part of the circadian cycle by causing drowsiness, and pantethine, which helps produce serotonin.

It is important to the operation of these supplements that all the ingredients are present in the proper amounts. Five ingredients were analyzed, as follows:

1) 5-Hydroxytryptophan (5-HTP)[5] can have side effects like dizziness, nausea,

1 All manufacturer claims from person communication with Connie Shoemaker of Sanesco International.

fatigue,[6] vomiting, and diarrhea in high doses[7] and may affect decision making under certain conditions.[8] 5-HTP has also been shown to be fatally toxic to dogs in extremely high doses.[9]

2) Melatonin, which can cause sleepiness or headaches.[10]
3) L-theanine, which appears to have no ill effects at high doses in rats.[11]
4) Glycine, which at high doses can cause vomiting, seizures, cyanosis, apnoea, oliguria, anuria and hypotension and can lead to death.[12]
5) Pantethine, which has no major side effects in the literature.

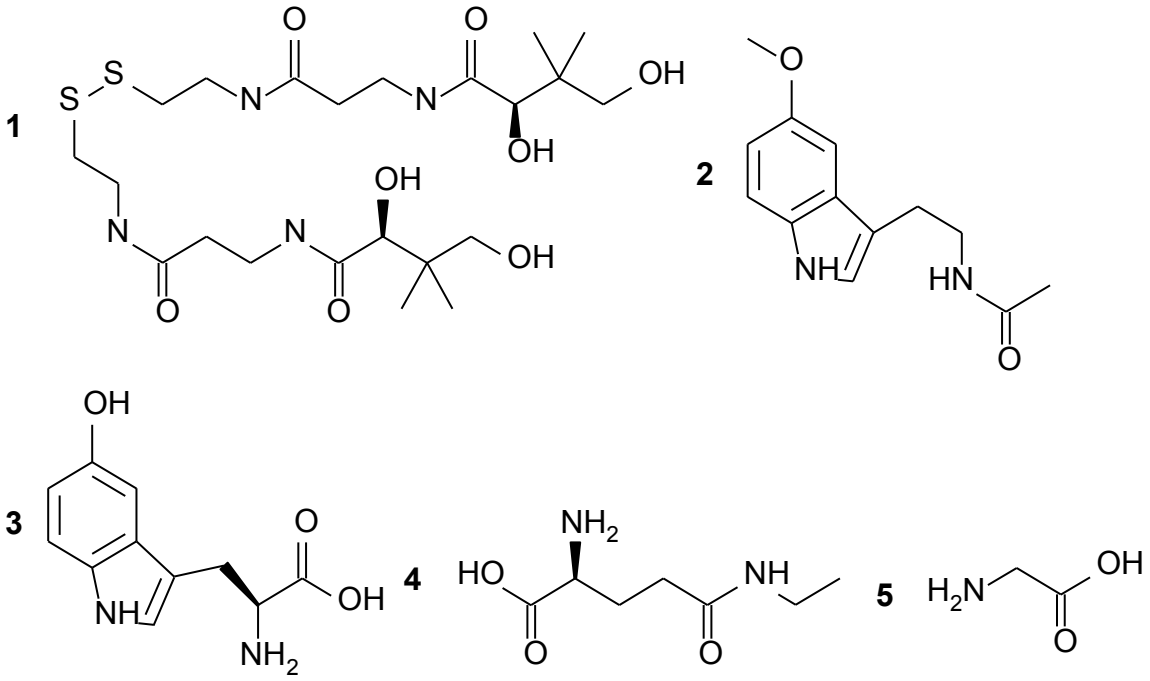
Even when there are no common side effects, there can be dangerous and unforeseeable consequences from an accidental overdose of "natural" dietary supplements, just as for any drug[13]. Therefore making sure that these ingredients do not exceed the prescribed amount is also an issue.

Statement of Objective

The specific aim of this project is to create a method by which the active ingredients of three Sanesco products, Lentra, Prolent, and Somni-TR, can be quantified, ensuring that there is neither a shortage of an important ingredient nor an overabundance of an ingredient which could have harmful side effects. The method employed to do this is reverse phase high performance liquid chromatography HPLC with UV detection. In this form of column chromatography, the mobile phase is forced through the stationary phase with a pump. This chromatographic method is often preferred over the older gravity-based column chromatography for analytical applications because of the ability to

use more densely packed stationary phases, which produce superior resolution. HPLC with a polar stationary phase and non-polar mobile phase is classified as “normal-phase” while “reversed-phase” uses a non-polar stationary phase and a polar, usually aqueous mobile phase. Reversed-phase is more convenient for most analysis because the column is more stable. A mainly aqueous mobile phase can be cheaper as well as less toxic than the organic solvents used in normal-phase HPLC. Selectivity can be improved by changing the polarity of the mobile phase or adding a second mobile phase. On a reversed-phase system, a less polar mobile phase may cause the nonpolar analytes to elute faster, thus decreasing the retention time. Adding a second, less polar mobile phase can achieve the same effect, and retention time of the analytes can be closely controlled by gradually changing the proportion of less polar solvent pumped through the column over the course of the run. As the polarity of the mobile phase changes, less polar solutes migrate through the column faster. This “gradient elution” improves the peak shape and decreases the retention time of non-polar analytes. This allows analytes of a wide range of structures and polarities to be quantified over a shorter run time. In the analysis of nutritional supplements, the analytes vary in structure as illustrated below. (Figure 1)

Figure 1. Structure of analytes: pantethine (1), melatonin (2), 5-HTP (3), L-theanine (4), glycine (5).



These compounds are all detectable with HPLC with UV detection, with each one incorporating a carboxyl, carbonyl or aromatic chromophore. This prevents the need for any derivatization. HPLC as a technique has good selectivity despite the similarities in structure between some of the analytes, although the smaller, more polar analytes such as glycine may come off too quickly because of low affinity with the stationary phase. A UV transparent buffer such as phosphate must be used as to not interfere with detection.

CHAPTER II

REVIEW OF LITERATURE

HPLC is an efficient method of quality control for all kinds of dietary supplements. Most such analysis uses reverse phase chromatography with octadecylsilanol attached to silica gel (C18) as the stationary phase. Other alkyl groups can be used, with longer alkyl in general providing increased retention of the analytes. Normal phase is used rarely, with unmodified silica gel the most common adsorbant. The mobile phase is typically a mix of water and an organic solvent such as methanol or acetonitrile. The pH of the aqueous solvent can be important depending on the acid or base character of the analyte. An acid component will be ionized at higher pH, which decreases retention time, so a pH of 2.5-5 is preferable. A lower pH also protonates residual silanols on the surface of the stationary phase. This prevents ion-exchange reactions from occurring between the acidic silanol and a basic amine functional group on the solute, which can cause peak tailing. A high pH can also destroy a column over time by dissolving the silica in the stationary phase. The usual method of controlling the pH is a phosphate buffer, although an acetate buffer can be used as long as UV detection below 230 nm is not required.[14] If the components of the mixture being analyzed are similar in structure and properties, then an isocratic method will suffice, but for mixtures such as as multivitamins with many different components a gradient is preferable[15,16]. A

slowly increasing percentage of organic mobile phase may enable the elution of substances with widely varying affinity for the stationary phase. Optical ultraviolet is the most common detection method, but other methods include coulometric detection (a highly sensitive form of detection which relies on the oxidation or reduction of the analyte and can be used on substances lacking a chromophore) or mass spectrometry.

The following examples are illustrative of the uses of HPLC in nutrient quantification. The caffeic acid derivatives in 16 commercial supplements of *Echinacea purpurea* were analyzed by HPLC in 2004, and a method of quantitation was developed for quantities of each derivative between 100 and 200 ng/mL.[17] Two separate HPLC methods were tried for quantification of melatonin and soy extracts in a formulation intended to treat menopausal symptoms; the results were in good agreement.[18] HPLC has been used to identify chemical fingerprints of raw plant extracts used in traditional medicine.[19]

Ascorbic acid, more commonly known as the water-soluble vitamin C, can be quantitated in both human plasma and a variety of fruit juices with a C-18 column at a wavelength of 265nm with a linearity of 0.05-100 μg/mL.[20] Vitamins B1, B6 and B12 have been determined in fruit juice and seafood with coulometric, electrochemical and ultraviolet detection at 283 nm.[21] Five forms of vitamin B6 have been separated isocratically and determined by coulometric detection[22]. A chromatographic method has been created for the simultaneous determination of seven B-complex vitamins using combined mass spectrometry and UV detection.[23] Simultaneous detection of B2, B3, B6,

caffeine and taurine in energy drinks was achieved with multi-wavelength scanning, with UV measurement of B3 and caffeine, fluorescence measurement of B2 and B6, and visible wavelength measurement of derivatized taurine.[24] Seven water-soluble vitamins have been measured in tarhana (a Turkish cereal food) by isocratic RP-HPLC with UV detection.[25] Superheated water chromatography using deuterium oxide has been used to quantify vitamins, allowing the use of NMR as a detector.[26]

Many of the active ingredients in these three supplements are amino acids. HPLC with UV-vis detection at 335 nm has been used to obtain derivitized amino acid profiles of proteins in vegetable oil[27]. 18 derivatized amino acids in olive, grapeseed, corn, avocado, peanut, hazelnut and soybean oil were separated with an acetonitrile/5 mM citrate buffer gradient within 50 minutes. HPLC has been compared to a micellar electrokinetic chromatography method[28] for the detection of melatonin, one of the ingredients of Somni-TR, and several of its metabolites. In this study, an HPLC method using a methanol/sodium acetate mobile phase (pH 5.1) on a C18 column and a diode array detector was compared to an MEKC method using phosphate buffer and SDS (pH 3.0) and also measuring with a DAD. The HPLC method proved to have higher accuracy in the quantitation of 5 μg/mL melatonin, although the MEKC method was much faster and had a lower limit of detection. HPLC with fluorescence detection has also been used to determine melatonin concentrations in bile and plasma[29]. 85 mM acetic acid with 0.1 mM disodium EDTA and 14% acetonitrile (pH 4.7) was used as the mobile phase here and melatonin eluted at about 9 minutes. Recoveries were 89-94% with a limit of

detection of 8 pg/mL. A method was developed for the determination of taurine in energy drinks with the 4-fluoro-7-nitrobenzofurazan derivatized taurine detected at 470 nm.[30] A disodium hydrogenphosphate/citric acid buffer (pH 5.4) was used with acetonitrile and the derivatized analyte eluted from the column within 6 minutes. The recovery using this method was 98.2-99.9% and the standard deviation of the results was 0.3-0.5%.

CHAPTER III

ANALYSIS OF NUTRITIONAL SUPPLEMENTS BY HPLC

Introduction

HPLC is a well-established method for the quantification of nutrients. One or more mobile phases is pumped through a column containing the stationary phase. Analytes in a sample injected into this system are retained according to polarity, and chromatograms are produced by measuring the time at which solutes are eluted from the column. Careful selection and manipulation of the mobile phase or mobile phases which are pumped through the column provides a way to customize the resolution of a given combination of compounds by changing the retention times. The nutritional supplements analyzed by HPLC in this project all contain compounds with chromophores which absorb in the ultra-violet spectrum and can be quantified with a UV detector.

Experimental

HPLC analysis was performed using an Agilent ZORBAX Eclipse XDB-C18 4.6 x 150 mm column (5 μm particles). The column was kept at 30ºC. Run time was 20 minutes with a gradient between mobile phase A (0.02M phosphate buffer, pH 2.5) and mobile phase B (acetonitrile). Flow rate was 2.0 mL/min.

Table 1. Mobile phase gradient: % mobile phase B vs run time.

Time	% Mobile Phase B
0-7	0-25
7-18	25-35
18-20	35-50
20-22	5

All peaks of interest eluted before 8 minutes and the remaining time was devoted to cleaning the column. Mobile phase A was made up with Nanopure Diamond ultrapure water and potassium phosphate monobasic. Solutions of pantethine, L-theanine, 5-HTP and glycine were prepared by dissolving in the aqueous mobile phase, while melatonin standard was made using 50% v/v ethanol and water as a solvent. Calibration curves were run using serial dilution of standards. Samples of nutritional supplements were provided by Sanesco International. Prolent and Lentra capsules were opened and the contents dissolved while Somni-TR tablets were ground with a mortar and pestle prior to dissolving. Samples of nutritional supplements were dissolved in mobile phase at a ratio of 1 capsule per 100 mL and filtered with vacuum filtration. Lentra and Somni-TR samples were run three times each and Prolent was run four times due to more erratic values. Measurements were taken at 200, 210, 225 and 280 nm for each sample. Absorbance is defined as the peak height in order to minimize error in case of poorly resolved peaks or peak tailing. Results were compared with ingredients listed on the back of the packaging. There were several ingredients not quantified including NSB, a proprietary blend of enzymes intended to increase ease of absorption.

Results

Standard curves (absorbance vs. concentration) for five analytes were measured. L-Theanine, pantethine and 5-HTP standard curves were created using chromatograms taken at 225 nm, melatonin at 280 nm, and glycine at 210 nm. Since all samples of nutritional supplements were dissolved in mobile phase at a ratio of 1 tablet per 100 mL of solvent, the projected concentration of each ingredient in solution is 1/100th of the listed quantity in milligrams per milliliter.

Table 2. Analytes, elution time, and preferred wavelength.

Analyte	Wavelength	Elution time	Supplements
L-theanine	225nm	.97 min	Lentra, Prolent (75 mg)
melatonin	280nm	7.5 min	Somni-TR (2 mg)
pantethine	225nm	5.7 min	Somni-TR (20 mg)
5-HTP	225nm	2.6 min	Prolent (50 mg)
glycine	210 nm	0.68 min	Prolent (150 mg)

L-Theanine

Prolent and Lentra are both advertised to include 75 mg of L-theanine. The standard curve encompasses a range of 0.125-1.00 mg/mL. When Prolent samples were run, the results proved to erratic with regard to L-theanine, with results ranging from 101 to 274 mg. The 274 mg value was an outlier, being almost twice the amount of the next highest value of 154 mg. All of these results were higher than the 75 mg indicated by the ingredient listing. It is unknown what could cause this degree of variation except a variation in the composition of the samples which were run. Lentra results appear more

consistent, averaging 80 mg with a standard deviation of 3 mg. The L-theanine standard exhibits a large peak at around 0.97 minutes accompanied by a much smaller peak at 0.87 minutes which is most likely a system peak, although it could also indicate a possible impurity or decomposition. This smaller peak is also exhibited on the chromatograms of Prolent and Lentra.

Figure 2. L-theanine standard (1 mg/mL) at 225 nm.

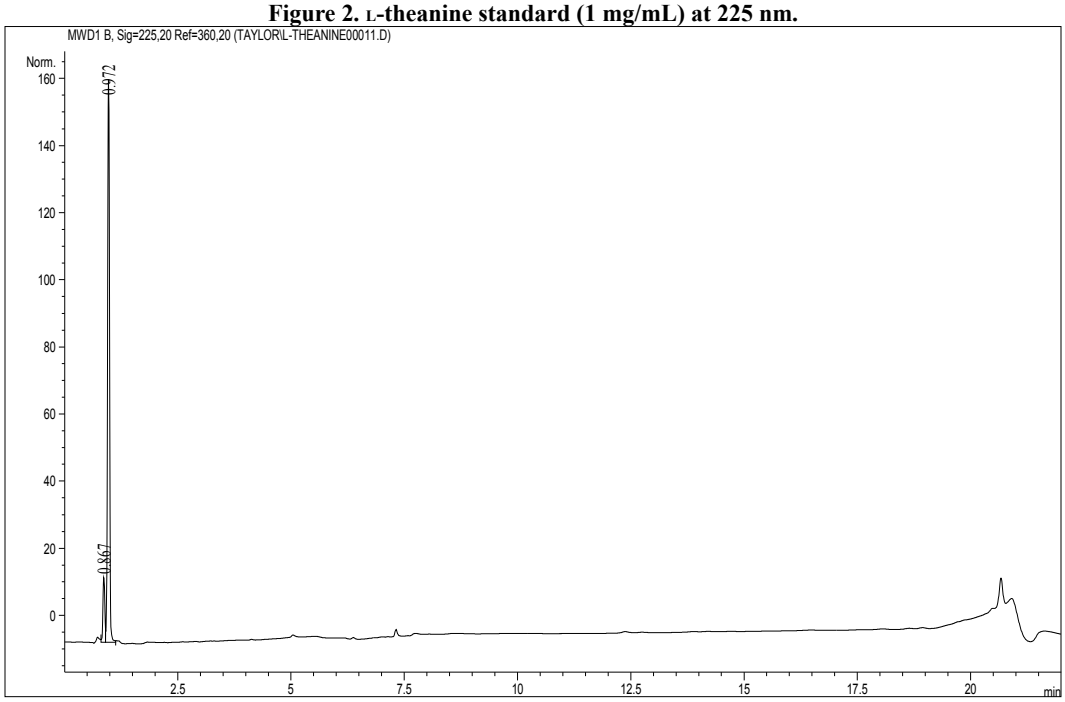
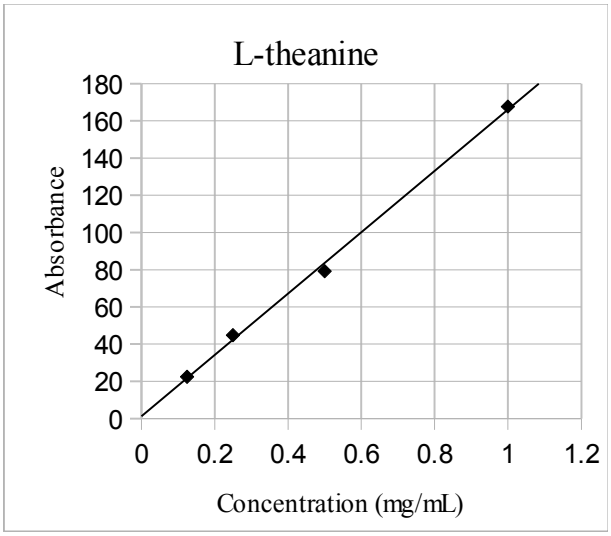


Figure 3. L-theanine standard curve. Absorbance = 165 * concentration+1.24, R^2 = 0.998



Melatonin

Somni-TR is advertised to include 2 mg of melatonin per tablet. Eight melatonin standards were run in concentrations ranging from 0.0125 mg/mL to 1.00 mg/mL. The average quantity found to be in the Somni-TR tablets was found to be 1.5 mg with a standard deviation of 0.4 mg.

Figure 4. Melatonin standard (0.125 mg/mL) at 280 nm.

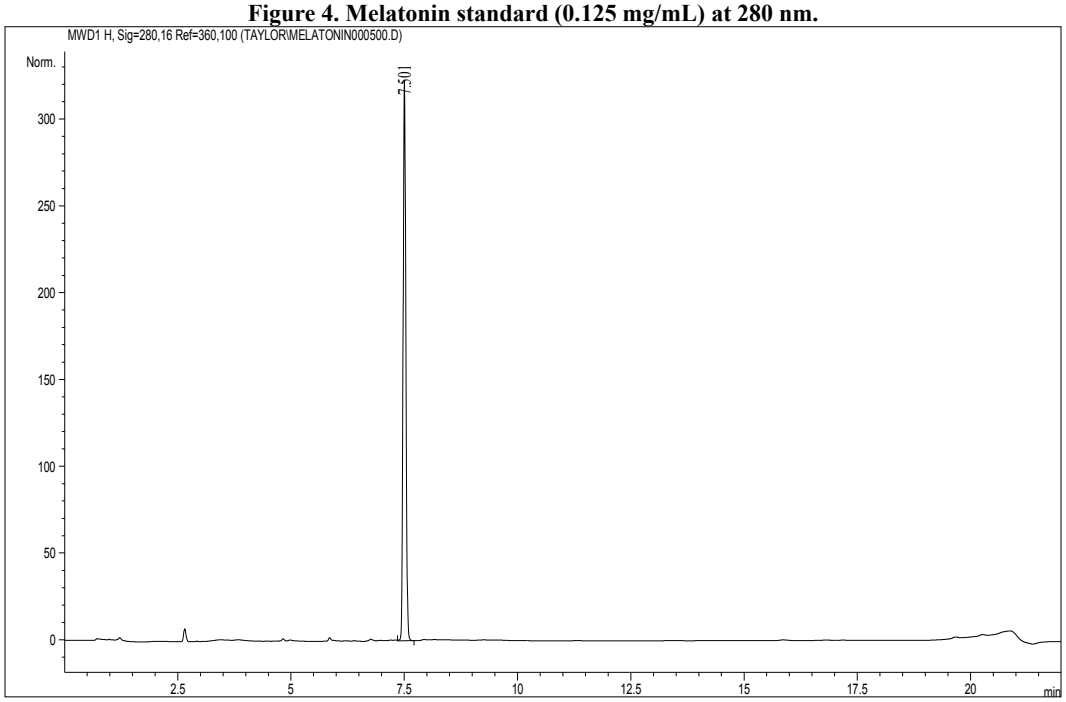
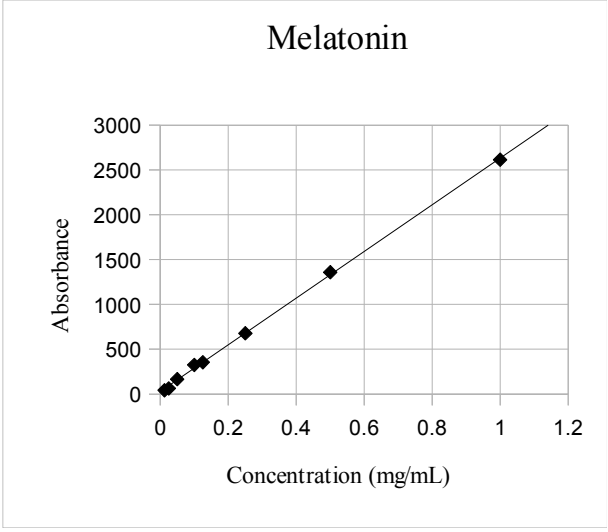


Figure 5. Melatonin standard curve. Absorbance = 260 * concentration + 29.3, R^2 = 0.999



Pantethine

Somni-TR is advertised to include 20 mg of pantethine per tablet. Four standards were run in in concentrations ranging from 0.050 to 0.805 mg/mL. Somni-TR samples show an average value of 25 mg per tablet with a standard deviation of 5 mg.

Figure 6. Pantethine standard (0.805 mg/mL) at 225 nm.

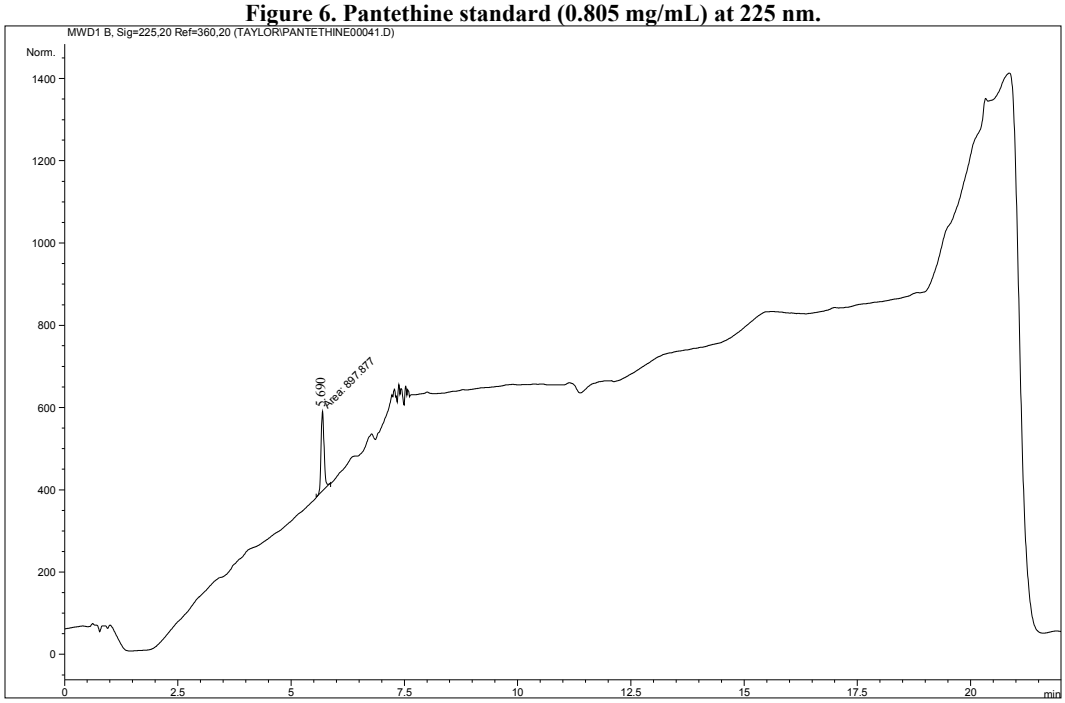
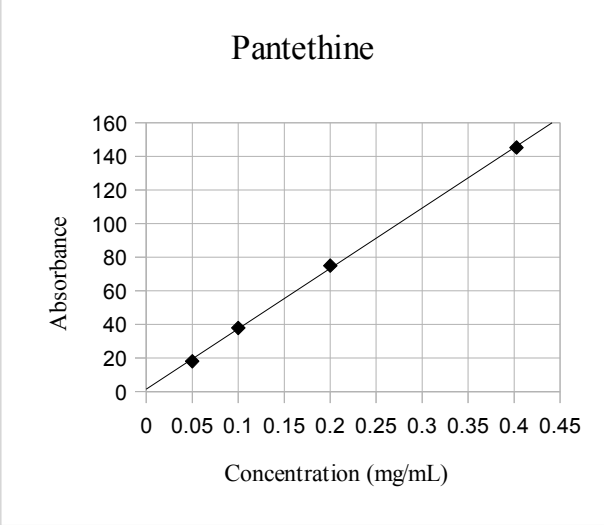


Figure 7. Pantethine standard curve. Absorbance = 359 * concentration + 1.50, R^2 = 0.999



Glycine

Prolent is advertised to include 150 mg of glycine per tablet. Four standards were run in concentrations ranging from 0.25-2.00 mg/mL. While the retention time at 0.68 seconds runs perilously close to the retention time of the void volume, the glycine peak was symmetrical. The Prolent samples had an average value of 210 mg of glycine with a standard deviation of 27 mg. Although the high value of glycine could be a result of overdosing, it is also possible that the high result is due to poor separation from an unknown peak eluting at 0.85 minutes or interference from other substances eluting at close to the void volume retention time.

Figure 8. Glycine standard (1 mg/mL) at 210 nm.

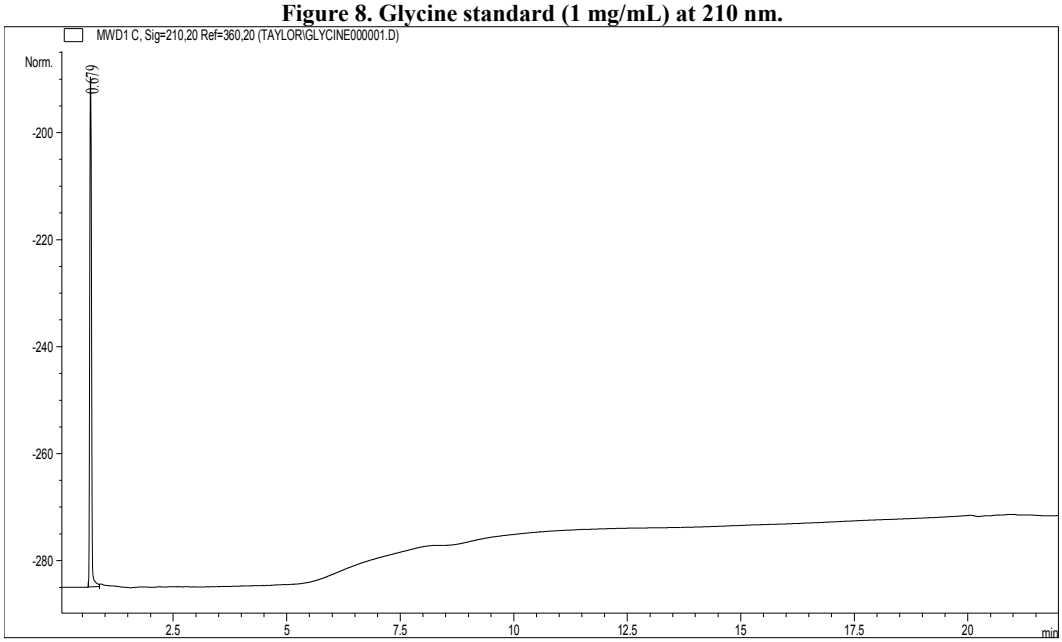
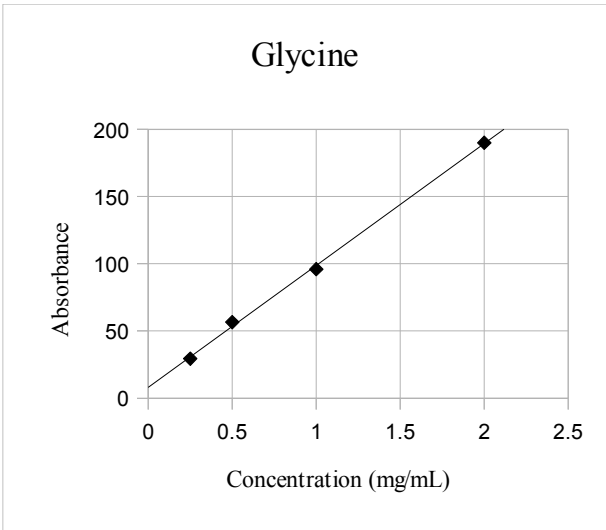


Figure 9. Glycine standard curve. Absorbance = 90.7 * concentration + 8.01, R^2 = 0.999



5-HTP

Prolent is advertised to include 50 mg of amino acid 5-HTP. Four standards were run in concentrations ranging from 0.125 to 1.00 mg/mL. Prolent samples showed an average concentration of 1.56 mg/mL, or 156 mg per tablet. The 5-HTP peak of Prolent samples shows some distortion, indicating that either the quantity of 5-HTP exceeds the linearity of the method, or that there is some interfering substance eluting at the same time. Another anomaly was in the 5-HTP standards, which showed well separated dual peaks, one at 2.6 minutes which coincided with the peaks in the Prolent chromatograms, as well as a second large peak at 0.85 minutes which did not appear with Prolent. For this reason, the latter peak was used for the standard curve. Both peaks show considerable tailing. The presence of the second peak in the standard indicates contamination or decomposition of the 5-HTP reagent, and may account for the abnormally high amount of 5-HTP appearing in Prolent.

Figure 10. 5-HTP standard (1 mg/mL) at 225 nm with peaks at 0.85 and 2.6 minutes.

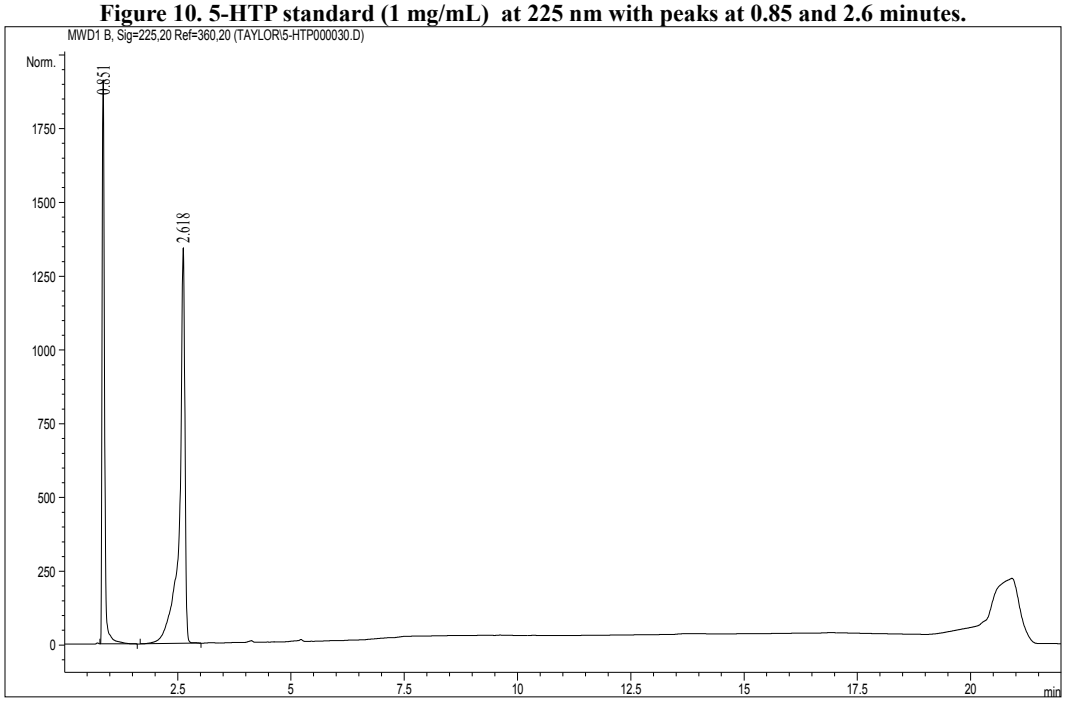


Figure 11. 5-HTP standard curve. A=1310C+50.4, R^2 = 0.999

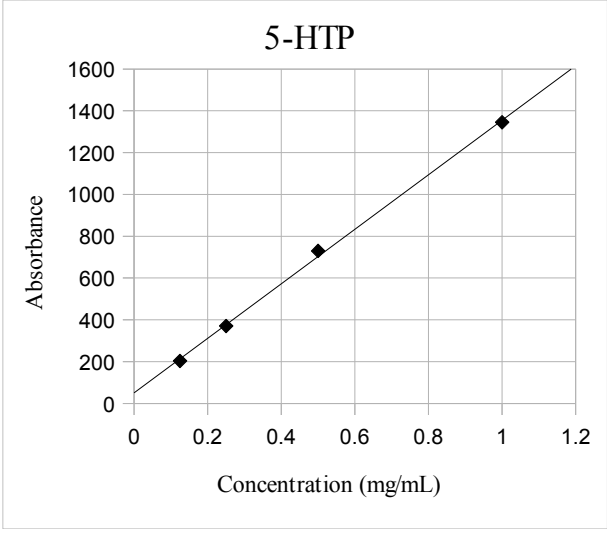


Figure 12. A chromatogram of Somni-TR at 225 nm.

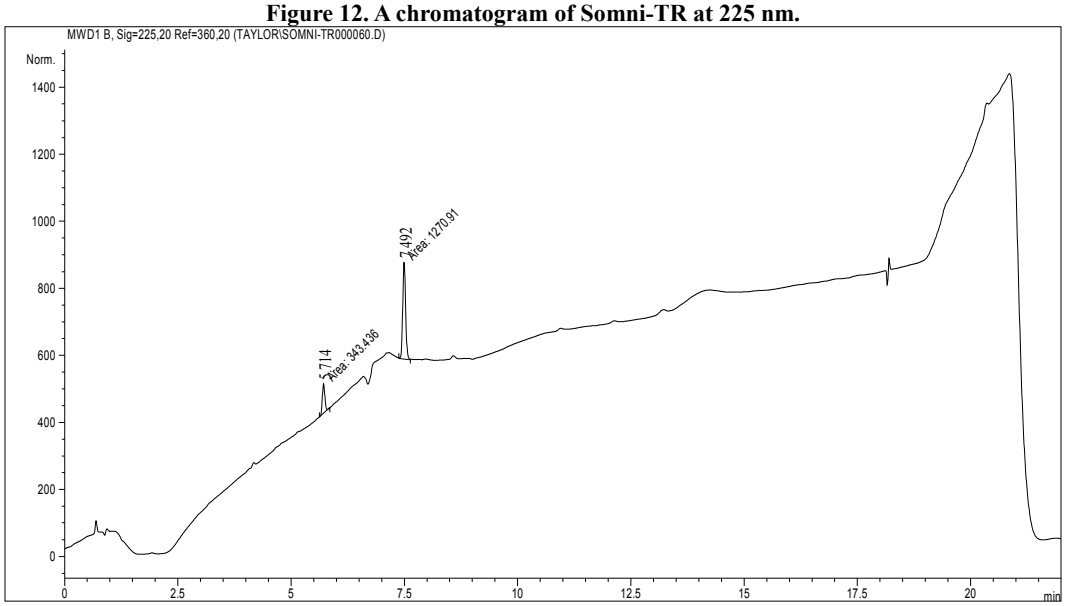


Figure 13. A chromatogram of Somni-TR at 280 nm.

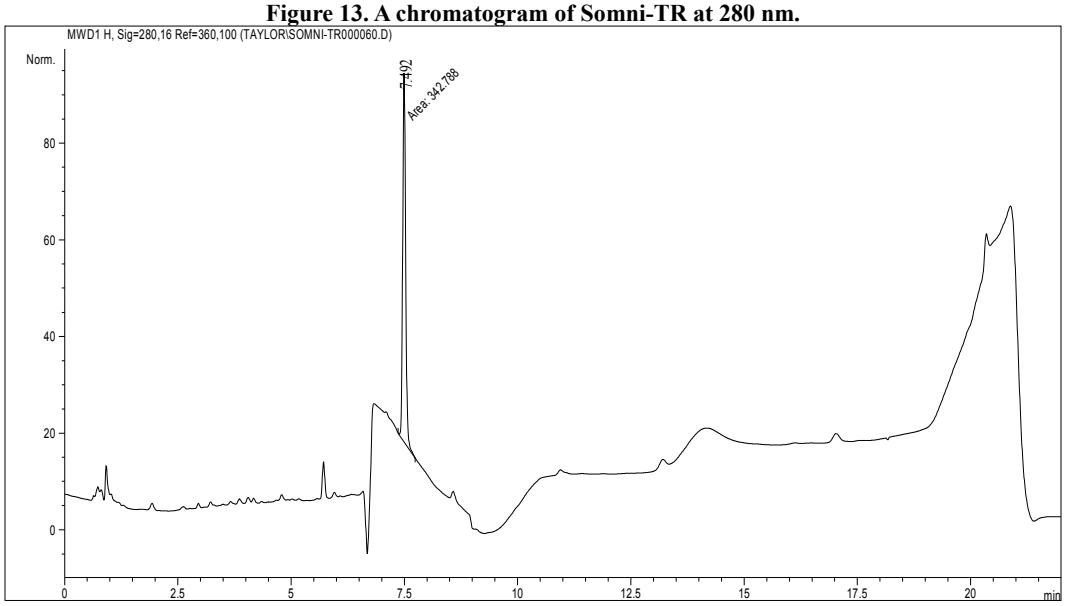


Figure 14. A chromatogram of Prolent at 210 nm.

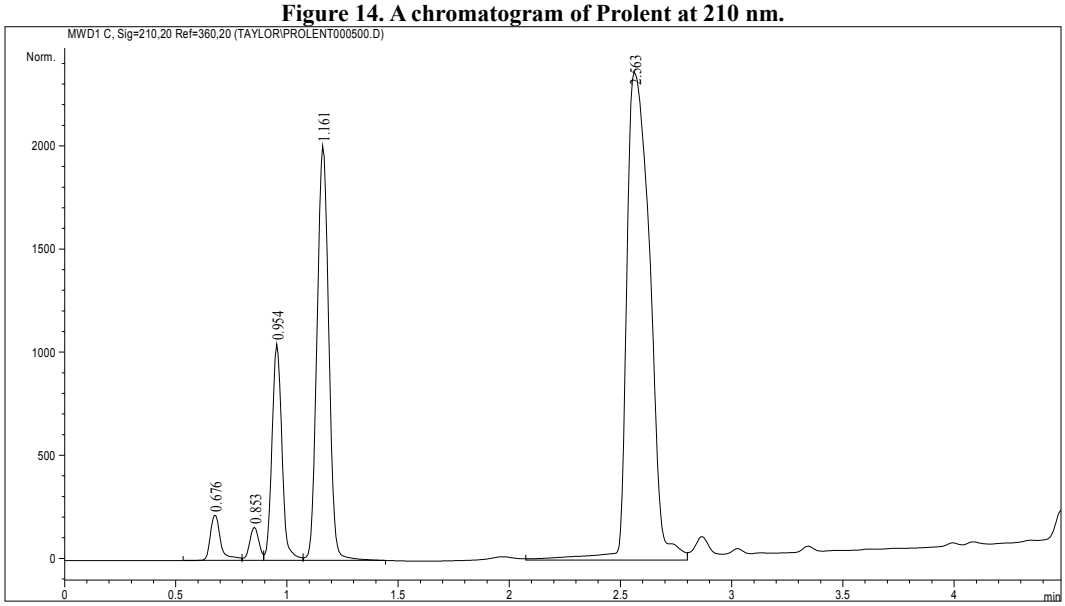


Figure 15. A chromatogram of Prolent at 225 nm.

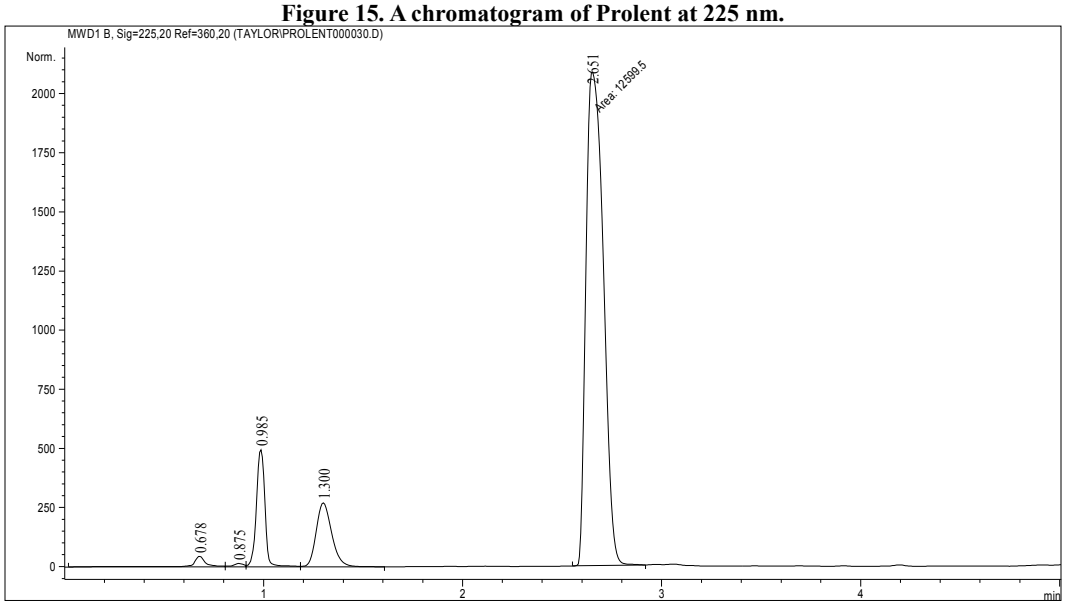
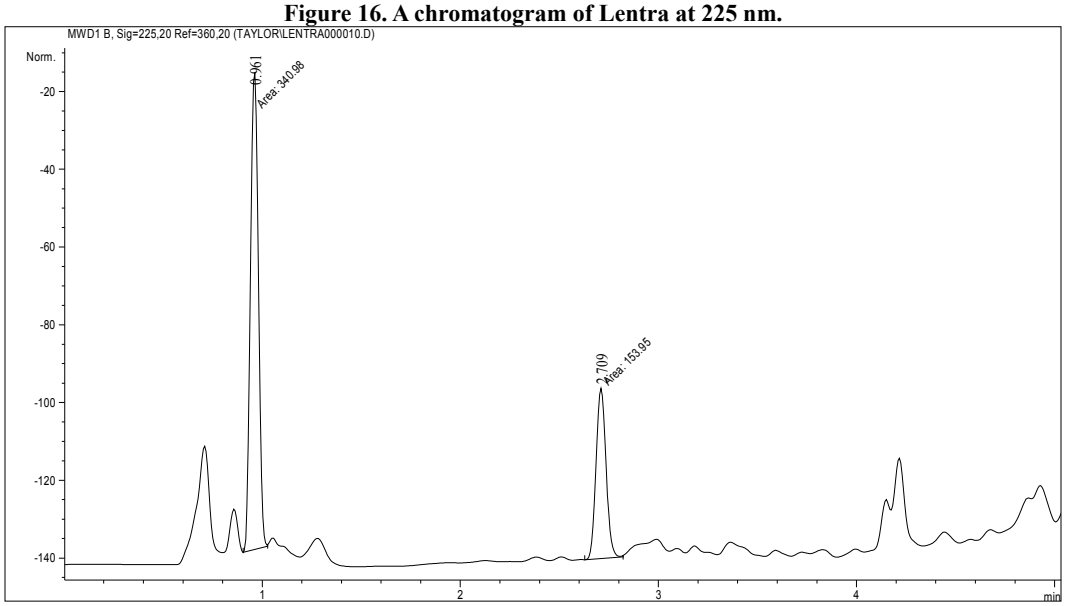


Figure 16. A chromatogram of Lentra at 225 nm.



CHAPTER IV

SYNTHESIS OF DIACETYLENE MONOMERS

Introduction

Chemosensors are used to detect chemical presences in the environment by converting a chemical interaction into a detectable signal. Humans and other animals are equipped with a certain number of natural chemosensors in order to detect changes within the body, offensive odors, or pleasant tastes. Artificial chemosensors are developed in order to detect specific chemicals or sometimes entire cells and viruses. Some examples of analytical methods used in artificial chemosensors are surface plasmon resonance and the quartz crystal microbalance. Both natural and artificial chemosensors consist of a receptor element which recognizes the analyte and a transducer which converts the recognition into a readable signal. One of the challenges involved in producing artificial receptors is tailoring the receptor to selectively respond to a specific analyte.

Statement of Objective

One means of creating selective receptors is to make a polymer mold of the analyte. Such a compound is called a molecularly imprinted polymer.

The objective of this study was to synthesize several diacetylenes which could be used to build polydiacetylene sensors. To this end, attempts were made using various methods to synthesize diacetylenes of different functional groups and chain lengths.

Review of Literature

The main benefit of molecularly imprinted polymers is their ability to recognize analytes for which there is no known receptor. The only restriction on substances which can be detected in this manner is that, since the analyte must be present during polymerization, it must be unable to either retard the polymerization or undergo polymerization itself. Another of the challenges of creating molecularly imprinted polymers is creating new polymers suitable for this purpose. The polymer must be stable under a variety of conditions and it must have functional groups able to interact with an analyte. The polymer must be able to either form a chemical bond with the analyte which can be broken easily enough to enable cleaning and reuse of the polymer sensor, or it must be able to hold the analyte in place by hydrogen bonds, Van der Waals forces, and pi-pi interactions. Non-covalent interactions are preferred because this makes removal of the template easier and because a greater variety of functional groups may be utilized.

Diacetylenes are a group of compounds which are notable for their optical characteristics in the polymerized form. Polydiacetylenes (PDAs) are brilliantly colored due to the excitation of π electrons along the conjugated backbone. The backbone of these polymers is tightly packed, and can produce two different optical states, a "blue" form with an absorption maximum of around 640 nm, and a "red" form with peak at around 540 nm. Additionally, the red form shows fluorescence with a large peak of around 640 nm and a small peak of around 560 nm, while the blue form shows no fluorescence whatsoever[31]. An irreversible transformation from the blue form to the red

can be induced with stimuli such as heating, mechanical stress[32], and the binding of target molecules or entire cells to tailored functional groups[33]. This transformation is the key to the potential usefulness of diacetylenes as sensory materials.

The cause of the optical transformation in polydiacetylenes are changes in the conformation of the polymer backbone.[34] Stress undergone by the polymer causes the rotation around the single bonds, thus breaking up the conjugation of the ene-yne bonds in the backbone.[35] With the shortening of the π electron delocalization length, the absorption maxima shifts to shorter wavelengths.

Polydiacetylene, made from 1,4-addition of the diacetylene monomers, can be structured as a bulk crystal, vesicles, or thin films. Most sensors use vesicles or thin films. For example, a polydiacetylene thin film with sialic acid residues was shown to be able to detect the influenza virus by binding to the viral hemagglutinin[31]. The inclusion of synthesized receptors embedded in PDA vesicles was used to selectively detect catecholamines in urine via fluorescence emissions.[36] Amphiphilic, water-soluble diacetylenes can be made to form vesicles with relative ease by addition of DMSO or DMF to an aqueous diacetylene solution and then polymerized by irradiation.

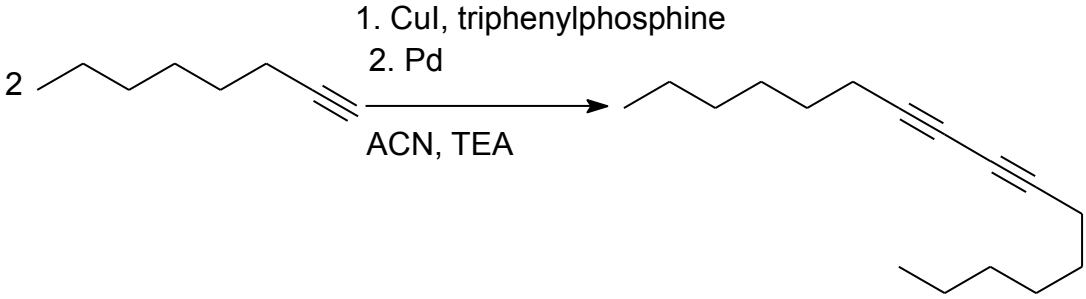
Diacetylene properties change according to composition of functional group and length of methylene spacer. Polymer conjugation length can vary according whether the monomer side chain contains an odd or even number of carbon atoms as well as the length of the side chain, with a longer side chain length and odd number of methylene groups contributing to higher polymerization efficiency.[37]

Synthesis of Diacetylene Monomers

Proton NMR spectra were used to analyze the results of all syntheses. Samples were prepared in d-water or d-chloroform and then run on a Jeol 500 MHz NMR spectrometer (JNM-ECA500). When impurities were found, products were purified using gravity column chromatography. As the fractions came off the column, they were collected and thin layer chromatography was used on each fraction in order to determine which contained the product. Mobile phase was eliminated by evaporating under reduced pressure and NMR was repeated.

1. Sonogashira coupling of 1-octynes[38]

Figure 17. Palladium catalyzed coupling of alkynes

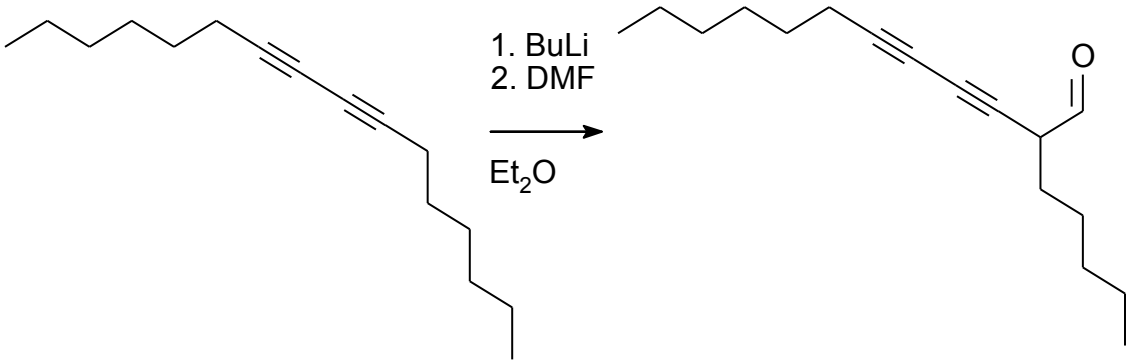


Acetonitrile (20 mL), cuprous iodide (0.114 g), triphenylphosphine (0.47 g), and triethylamine (30 mL) were mixed with 1-octyne in a Schlenk flask. This mix was degassed by freezing with liquid nitrogen, then placed under vacuum while thawing. After thawing was finished, the mixture was refrozen and the thawing was repeated. Once this was accomplished, bis(triphenylphosphine)palladium(II) dichloride was added. The flask was placed under a nitrogen stream and allowed to sit for 24 hours.

The solution was washed with ether into a round-bottom flask, then solvent was removed by evaporation. The remaining solid was dissolved in more ether, and then washed twice with HCl and once with tap water. The ether was dried with sodium sulfate and solvent was evaporated again.

2. The addition of aldehyde functional group to diacetylene

Figure 18. Addition of aldehyde to diacetylene with butyllithium



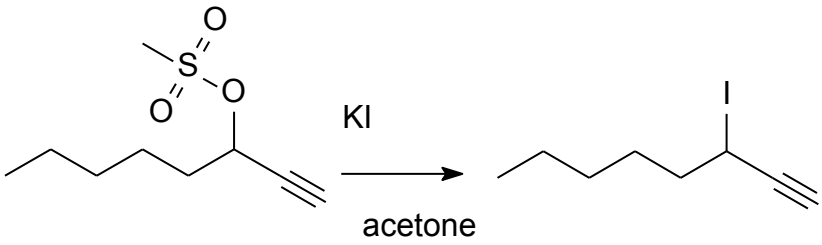
Diacetylene from the previous step was dissolved in ether and chilled with liquid nitrogen under streaming nitrogen gas. N-Butyllithium (4 mL) was added dropwise over the course of 10 minutes in order to remove the acidic protons. Then the flask was allowed to warm up and a greenish brown color was observed. Solution was added to 2.5 mL chilled dimethylformamide by cannulating. The mixed solution was left for 24 hours, after which a dark brown color was observed.

Product was washed once with hydrochloric acid to remove leftover amine and twice with tap water to remove dimethylformamide. The remaining solvent was

evaporated off. Sample was purified by column chromatography using 5% ethyl acetate and 95% hexanes. The synthesis of this compound either did not go to completion or was not fully purified as NMR spectra did not match the literature.[39]

3. Replacement of methyl sulfonyl with iodide group[40]

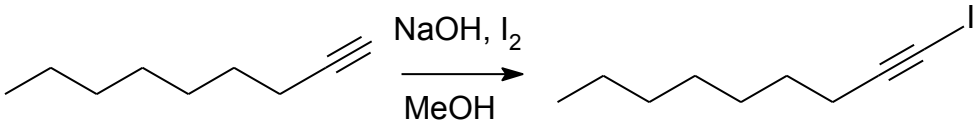
Figure 19. Substitution of sulfonyl with iodide



Potassium iodide (2.86 g) was dissolved in acetone, then of 1-octyne 3-sulfone (3.52 g) was added. This mixture was wrapped in foil and left overnight. The solvent was then evaporated off and the remaining solid dissolved in ether and washed with water. NMR showed that this reaction did not go to completion, so the solution was redissolved in acetone and potassium iodide was added again, then the mixture was heated to 100 degrees in an oil bath under reflux. Solution was dissolved in ether and washed with water. NMR did not match the predicted spectra.

4. Iodination of nonyne

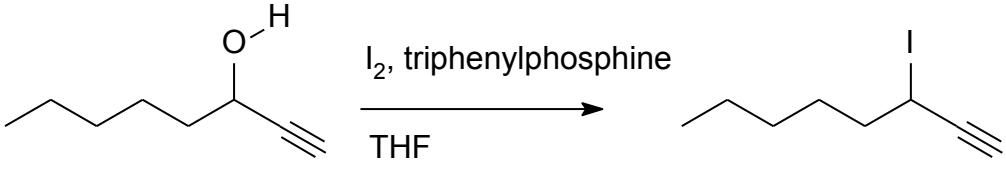
Figure 20. Base catalyzed iodination of terminal alkyne with sodium hydroxide



Sodium hydroxide (3 g) and iodine (2.01 g) was dissolved in methanol, then of nonyne (1.0 g) was added dropwise to the solution. Solution was allowed to stir for 24 hours, and then all but 25 mL of the solvent was evaporated off. Sodium thiosulfate was added to the dark brown solution to remove excess iodine. The liquid was washed three times with ether. The ether portion was dried over sodium sulfate and then evaporated. The remaining solid was dissolved in hexanes to remove emulsions, then evaporated again. NMR did not match the predicted spectra.

5. The iodination of 3-octynol[41]

Figure 21. Substitution of alcohol with iodide

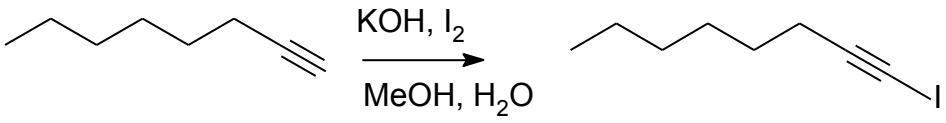


To start the reaction triphenylphosphine (25 g) and iodine (24 g) were dissolved in 400 mL tetrahydrofuran. 3-Octynol (10 g) was added dropwise to this solution and left for 24 hours. The tetrahydrofuran was then evaporated off and the remaining solid was dissolved in 200 mL diethyl ether and stirred for 30 minutes. The solid, consisting of

triphenylphosphine and iodide salts, was filtered out with glass wool and the brown liquid portion was washed with sodium thiosulfate until all color was gone. Then the ether solution was washed with water, dried over sodium sulfate, and evaporated until dry. NMR did not match the predicted spectra.

6. Iodination of octyne[42]

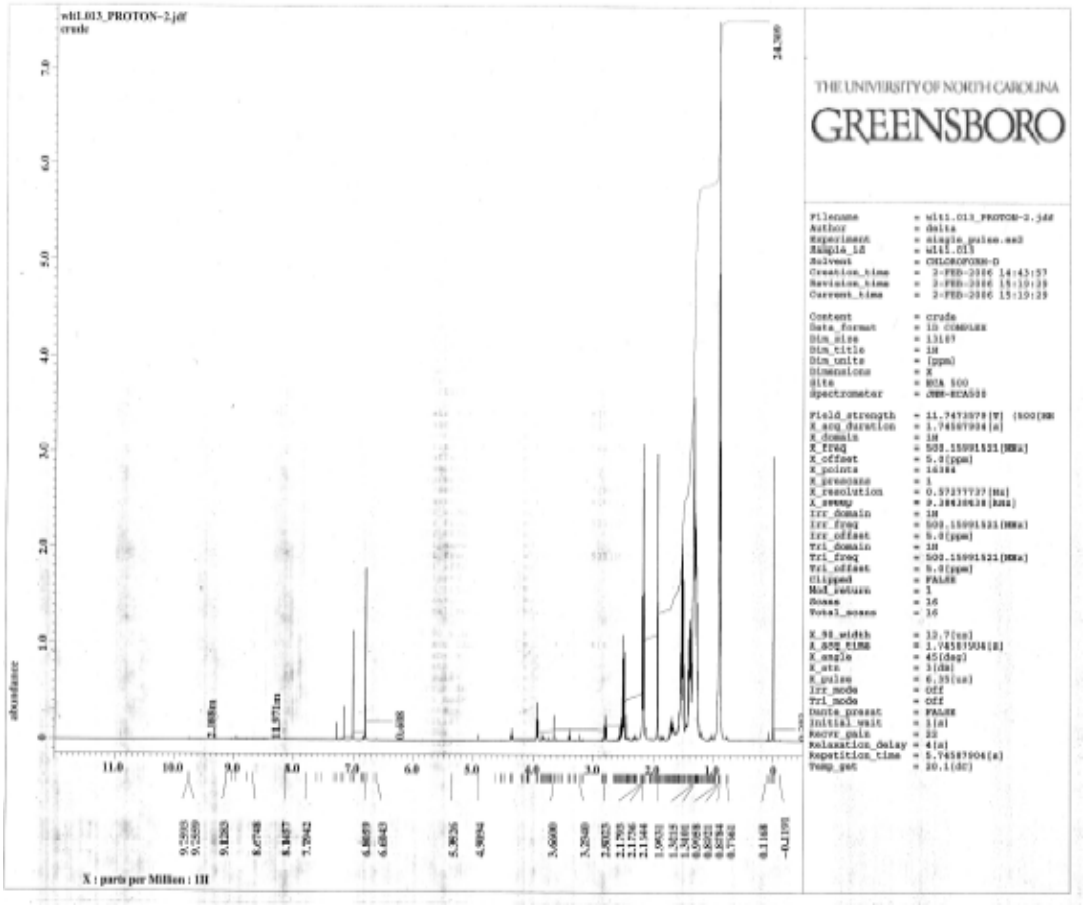
Figure 22. Base catalyzed iodination of terminal alkyne with potassium hydroxide



Potassium hydroxide (6.9 g) was dissolved in 50% v/v water and methanol. Iodine (15.61 g) was added slowly, producing a white precipitate, followed by octyne (13.16 g) dropwise over the course of ½ hour. Solution was left to sit overnight.

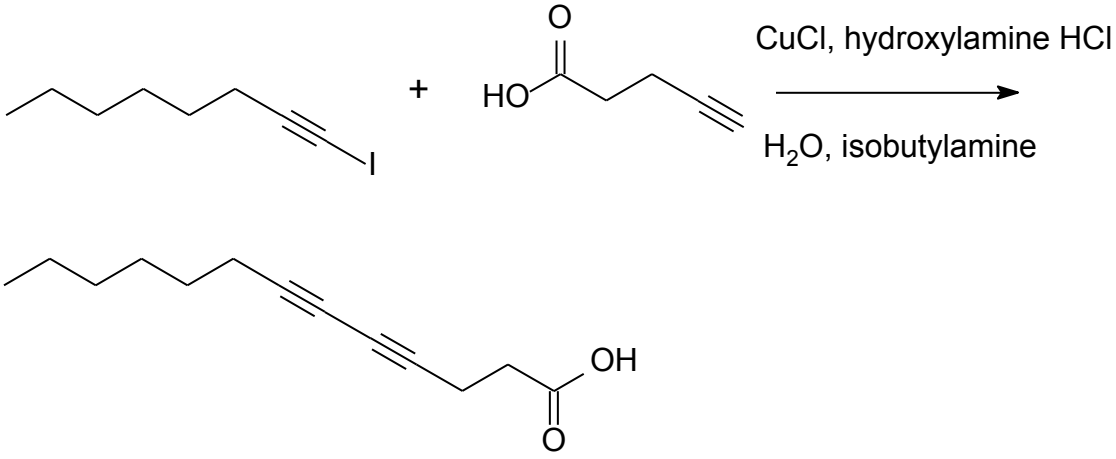
The solution was washed 3 times with hexanes, which produced a filmy layer between the two phases. This was filtered with cotton. Leaving the hexane solution out overnight caused it to turn a pink color, which is removed by washing with water and then evaporating the solvent. The remaining product is a yellowish brown color. During the evaporation of solvent after TLC, the solution fell into the water bath and had to be re-extracted. The yield of this product was 2.644g. NMR of the unpurified product was similar to the literature.[43]

Figure 23. NMR spectrum 1-iodo-1-octyne



7. Coupling iodoalkyne to carboxylic acid[44]

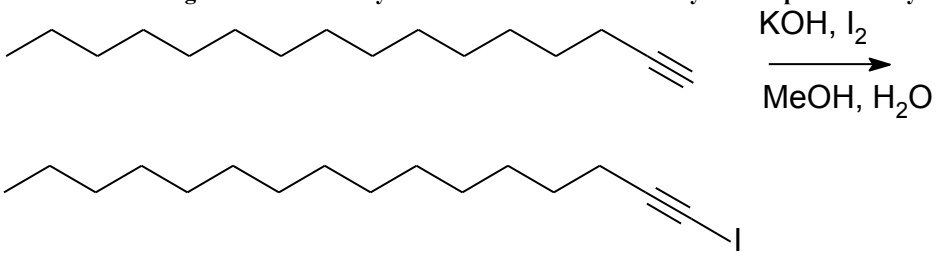
Figure 24. Copper catalyzed coupling of alkyne and iodoalkyne



Pentynoic acid (1.04 g) was dissolved in aqueous potassium hydroxide. This solution was then mixed with a solution of cuprous chloride in isobutylamine and water, and hydroxylamine hydrochloride (0.017 g) was added. The 1-iodooctyne was dissolved in a 1:1 mixture of methanol and ether, and added dropwise to the cuprous chloride solution alternately with a solution of 10% hydroxylamine hydrochloride solution. The solution was allowed to stir for 15 minutes and then was acidified with hydrochloric acid to ~pH 2. The solution was then washed three times with ether, which was then washed with sodium thiosulfate. NMR did not match the predicted spectra.

8. Iodination of hexadecyne[42]

Figure 25. Base catalyzed iodination of terminal alkyne with potassium hydroxide

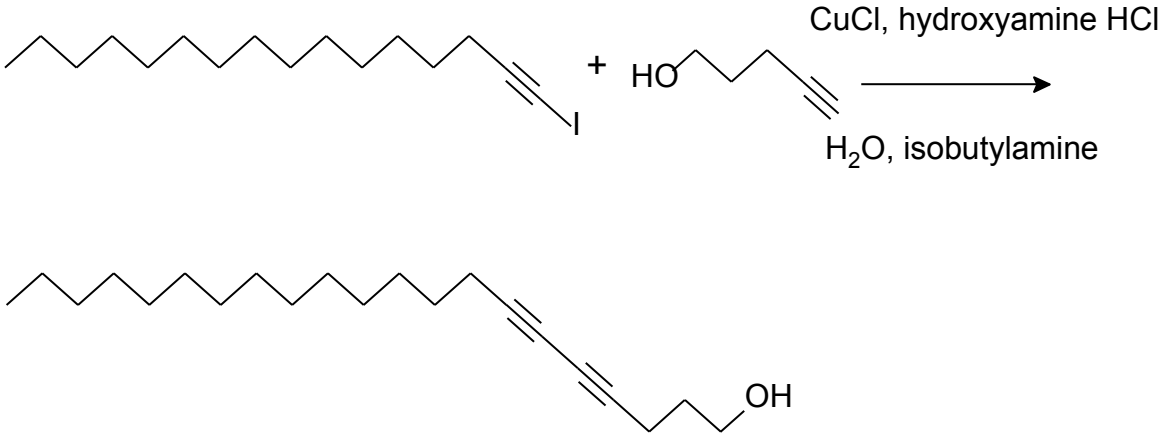


Iodine (8 g) and potassium hydroxide (3.5 g) were added slowly to a mixture of 150 mL H_2O and 130 mL MeOH. Hexadecyne (16.0 g) was added dropwise over the course of ½ hour and left for four days, after which more iodine was added. Solution was left overnight for another two days.

The solution was then washed 4 times with hexanes. The organic phase was saved and washed twice with sodium thiosulfate, once with sodium bicarbonate and once with water. The organic phase was then dried over sodium sulfate.

9. The coupling of 1-iodohexadecyne with 1-hexyn-6-ol[44]

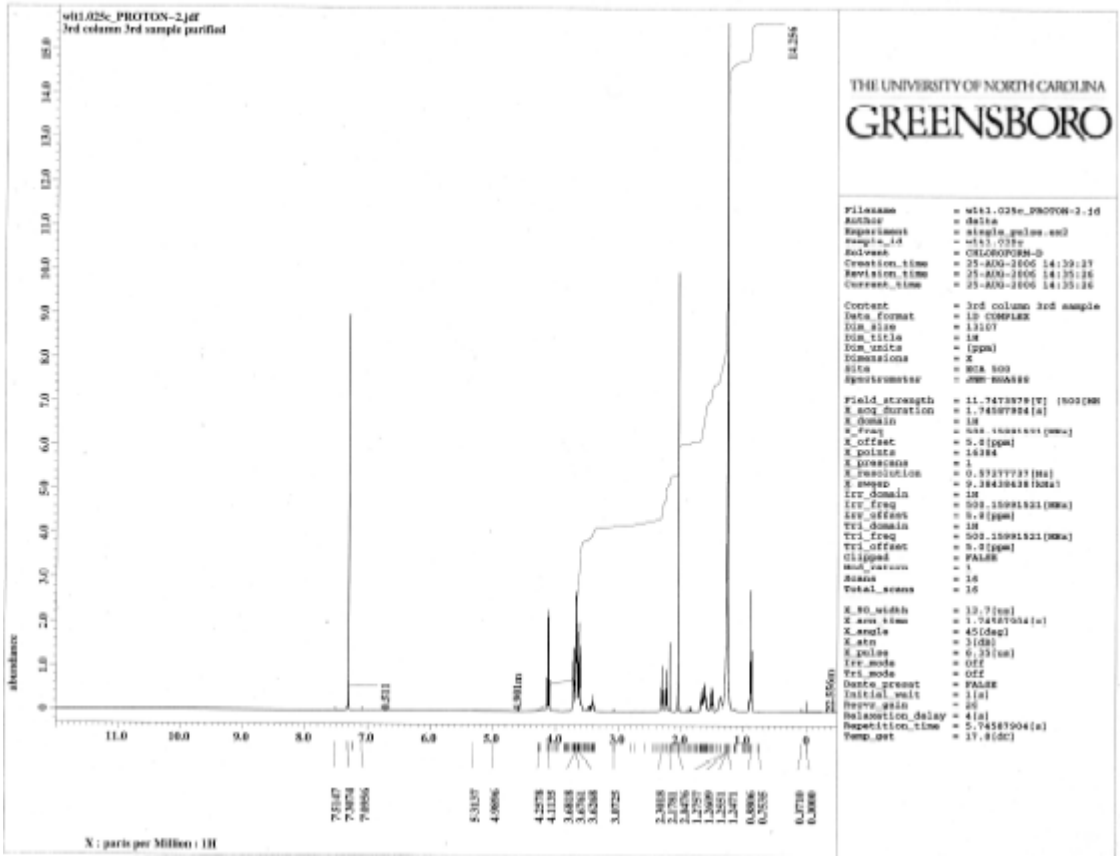
Figure 26. Copper catalyzed coupling of iodoalkyne with terminal alkyne



A sample of iodohexadecyne (2.1 g) from the previous reaction was dissolved in 20 mL 50% v/v methanol and ether. Hexynol (0.6 g) was dissolved in potassium hydroxide solution. Cuprous chloride (0.2 g) was dissolved in 50% v/v isobutyl amine and water, producing a dark blue solution. The alcohol and cuprous chloride solutions were then mixed, and hydroxylamine hydrochloride (0.0275 g) were added, causing the blue cuprous chloride solution to turn green. An additional 25 mL solution of 10% hydroxylamine hydrochloride was made up and added to the cuprous chloride solution dropwise, alternating with iodide solution. The solution was allowed to sit for 20 minutes, then acidified to pH 3 with hydrochloric acid. This caused a yellow precipitate to form. Solution was washed 3 times with hexanes and the yellowish colored organic phase washed with sodium thiosulfate. After the solvent was evaporated, a yellow solution was left along with some of the yellow precipitate. This procedure was repeated a second time

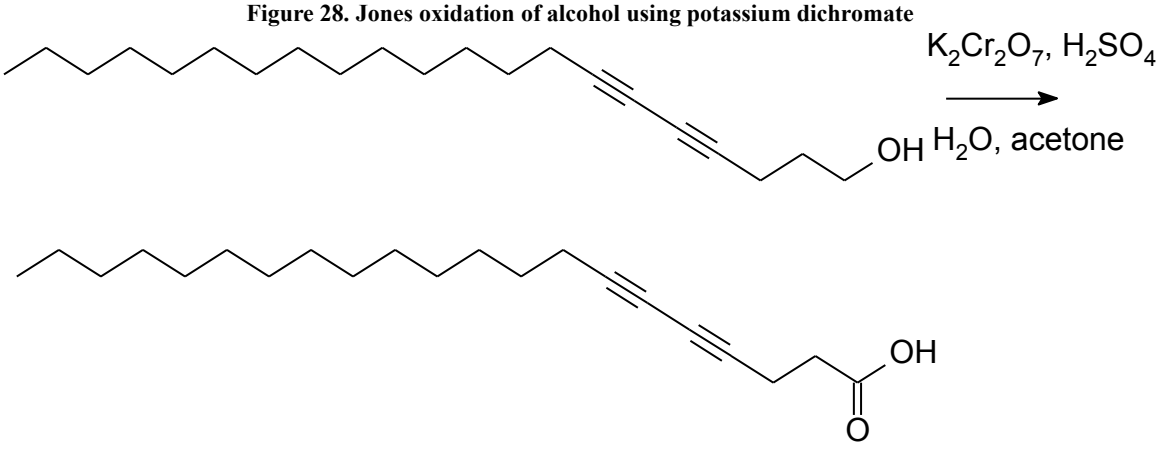
with another iodohexadecyne (2.1 g). Once the procedure was worked up, the remaining 14.5 g of reactant was reacted with 4.12 g hexynol. The product was then analyzed with NMR and some impurities were detected, so it was purified with column chromatography using a solvent of 20% ethyl acetate in hexanes, resulting in two different fractions with slightly different NMR spectra. Spectra were similar to predicted results although still containing impurities.

Figure 27. NMR spectrum of the coupled product of 1-iodohexadecyne and 1-hexyn-6-ol.



10. Jones oxidation of diacetylene alcohol

Figure 28. Jones oxidation of alcohol using potassium dichromate



The next step was to oxidize the alcohol into a carboxylic acid. This was first done on the second sample of product from the column in the previous step, which was about 0.65 g of reactant. The product was dissolved in acetone. Potassium dichromate (0.84 g) and concentrated sulfuric acid (3.0 g) were dissolved in a 1:2 mixture of water and acetone. This solution was then added to the alcohol solution and left overnight. In the morning the solution was a green color with white precipitate, which was filtered out from the solution with glass wool. Methanol was added to dispose of the excess oxidizer. After 1 ½ hours, sodium bicarbonate was added to neutralize the sulfuric acid. Blue precipitate formed. This solid was separated out with vacuum filtration, then the solvent was evaporated, leaving a yellow oil. The yield from this portion of the alcohol was extremely low.

The procedure was repeated on the second alcohol sample, with the exception that the white precipitate was saved and dissolved in chloroform.

CHAPTER V

CONCLUSIONS

HPLC Method Development

A method was developed for the quantification of five analytes in nutritional supplements by reverse phase HPLC. Standard curves were created and samples of nutritional supplements were analyzed for ingredients, with the results compared to the information listed on the ingredient labels. Of the three products, Somni-TR results conform the most closely to the ingredient label. Somni-TR also showed the best separation of peaks. Measured levels of L-theanine in Lentra were also similar to the quantity listed on the label. Prolent was more problematic, with results for L-theanine appearing highly erratic and glycine and 5-HTP to be greater than that officially listed as ingredients. The most troubling of these results is 5-HTP, although the double-peak irregularity of the 5-HTP standard makes these high results questionable. There is a possibility that the glycine peak overlaps with some unknown substance. The purity of this peak could be checked by taking the ratio of absorbance at two different wavelengths. The ratio could then be checked against the wavelength ratio of a glycine standard.

Diacetylene Synthesis

Three attempts to synthesize a diacetylene were made and seven attempts to functionalize a diacetylene or acetylene monomer were made. NMR results showed that there were problems with synthesizing and purifying the products. Although most of the NMR results did not match the literature values, they contribute to the total knowledge of synthesis of diacetylene products.

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